

LAG3 [CAL26]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3213-070318

BIOCARE
M E D I C A L

Catalog Number:	ACI 3213 A, B	API 3213 AA
Description:	0.1, 0.5, ml concentrated	6.0 ml, prediluted
Dilution:	1:100	Ready-to-use
Diluent:	Van Gogh Yellow	N/A

Intended Use:

For In Vitro Diagnostic Use

LAG3 [CAL26] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of LAG3 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

LAG-3 (Lymphocyte Activation Gene 3) is a surface receptor expressed on activated T cells, an exhaustion marker with immunosuppressive activity (1). Major histocompatibility complex class II (MHC-II) is a ligand for LAG-3; additional ligands (e.g., LSECtin and galectin-3) have also been identified (1,2). Regulatory T cells (Tregs) expressing LAG-3 have enhanced suppressive activity, whereas cytotoxic CD8+ T cells expressing LAG-3 have reduced proliferation rates and effector cytokine production in cancer and autoimmune diabetes (3,4). LAG-3+ tumor-infiltrating lymphocytes (TILs) have been reported in melanoma, colon, pancreatic, breast, lung, hematopoietic, and head and neck cancer patients (5-11), in association with aggressive clinical features. Antibody-based LAG-3 blockade in multiple cancer mouse models restores CD8+ effector T cells and diminishes Treg populations, an effect enhanced when combined with anti-PD-1 (12). Recent studies in a metastatic ovarian cancer mouse model showed that LAG-3 blockade leads to upregulation of other immune checkpoints (PD-1, CTLA-4, and TIM-3), and combination therapy targeting LAG-3, PD-1, and CTLA-4 increases functional cytotoxic T cell levels while reducing Tregs and myeloid-derived suppressor cells (13,14). Multiple pre-clinical and clinical studies are testing antagonistic LAG-3 agents in combination with anti-PD-1 and/or anti-CTLA-4 therapy (12-15). In view of the activating properties of soluble secreted LAG-3, a soluble agonist LAG-3 antibody (IMP321) was tested in advanced solid malignancies as a single agent (15), and demonstrated sufficient tolerability and efficacy to warrant advancement to phase II (16).

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, an enzyme labeled polymer is added to bind to the primary antibody. This detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Rabbit monoclonal

Species Reactivity: Human

Clone: CAL26

Isotype: IgG1

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.

Epitope/Antigen: Synthetic peptide derived from a region of the LAG3 protein

Cellular Localization: Membrane/cytoplasm/Golgi

Positive Tissue Control: Tonsil

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

Storage and Stability:

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations (intelliPATH and manual use):

Peroxide Block: Block for 5 minutes with Biocare's Peroxidized 1.

Pretreatment: Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 minutes at RT with a secondary-conjugated polymer.

Protocol Recommendations (intelliPATH and manual use) Cont'd:

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB -OR- Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Protocol Recommendations (Ventana BenchMark ULTRA Slide Staining System):

API3213 is compatible for use with the Ventana BenchMark ULTRA Slide Staining System. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Template/Detection: OptiView DAB IHC

Pretreatment Protocol: CC1 64 minutes

Peroxidase: Pre Primary Peroxidase Inhibitor

Primary Antibody: 32 minutes, 36°C

Technical Note:

This antibody, for intelliPATH and manual use, has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

Performance Characteristics:

Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (17)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (18)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

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References:

- Anderson AC, *et al.* Lag-3, tim-3, and TIGIT: Co-inhibitory receptors with specialized functions in immune regulation. *Immunity*. 2016; 44:989-1004.
- Kouo T, *et al.* Galectin-3 Shapes Antitumor Immune Responses by Suppressing CD8+ T Cells via LAG-3 and Inhibiting Expansion of Plasmacytoid Dendritic Cells. *Cancer Immunol Res*. 2015; 3:412-23.
- Bettini M, *et al.* Cutting edge: accelerated autoimmune diabetes in the absence of LAG-3. *J Immunol*. 2011; 187:3493-8.
- Williams JB, *et al.* The EGR2 targets LAG3 and 4-1BB describe and regulate dysfunctional antigen-specific CD8+ T cells in the tumor microenvironment. *J Exp Med*. 2017; 214:381-400.
- Shapiro M, *et al.* Lymphocyte activation gene 3: a novel therapeutic target in chronic lymphocytic leukemia, *Haematologica*. 2017; 102:874-82.
- Tassi E, *et al.* Early effector, lymphocytes coexpress multiple inhibitory receptors in primary non-small cell lung cancer. *Cancer Res*. 2017; 77:851-61.
- Bottai G, *et al.* An immune stratification reveals a subset of PD-1/LAG-3 double-positive triple-negative breast cancers, *Breast Cancer Res*. 2016; 18:121.
- Deng WW, *et al.* LAG-3 confers poor prognosis and its blockade reshapes antitumor response in head and neck squamous cell carcinoma. *Oncoimmunology*. 2016; 5:e1239005.
- Liosa NJ, *et al.* The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discovery*. 2015; 5:43-51.
- Meng Q, *et al.* Expansion of tumor-reactive T cells from patients with pancreatic cancer. *J Immunother*. 2016; 39:81-9.
- Demeure CE, *et al.* T Lymphocytes infiltrating various tumour types express the MHC class II ligand lymphocyte activation gene-3 (LAG-3): role of LAG-3/MHC class II interactions in cell-cell contacts. *Eur J Cancer*. 2001; 37:1709-18.
- Woo SR, *et al.* Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*. 2012; 72(4):917-27.
- Huang RY, *et al.* LAG3 and PD1 co-inhibitory molecules collaborate to limit CD8+ T cell signaling and dampen antitumor immunity in a murine ovarian cancer model. *Oncotarget*. 2015; 6:27359-77.
- Huang RY, *et al.* Compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer. *Oncoimmunology*. 2017; 6:e1249561.
- Baumeister SH, *et al.* Coinhibitory pathways in immunotherapy for cancer. *Annu Rev Immunol*. 2016; 34:539-73.
- Legat A, *et al.* Vaccination with LAG-3Ig (IMP321) and Peptides Induces Specific CD4 and CD8 T-Cell Responses in Metastatic Melanoma Patients--Report of a Phase I/IIa Clinical Trial. *Clin Cancer Res*. 2016 Mar 15;22(6):1330-40.
- Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
- Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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Table 1: Sensitivity and specificity were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Bladder Cancer	0	10
Breast Cancer	0	16
Colon Cancer	0	17
Lung Cancer	0	18
Ovarian Cancer	0	10
Prostate Cancer	0	11
Renal Cancer	0	10
Melanoma	0	3

Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebellum	0	1
Cerebral Cortex	0	1
Pituitary	0	1
Adrenal Gland	0	1
Thymus	1	1
Tonsil	3	3
Thyroid	0	1
Esophagus	0	1
Stomach	0	1
Small Intestine	0	1
Colon	0	1
Appendix	1	1
Pancreas	1	1
Spleen	1	1
Ovary	0	1
Cervix	0	1
Endometrium	0	1
Fallopian Tube	0	1
Placenta	0	1
Kidney	0	11
Bladder	0	1
Urethra	0	1
Breast	0	3
Prostate	0	5
Testis	0	1
Myocardium	0	1
Smooth Muscle	0	1
Skeletal Muscle	0	1
Lymph Node	1	1
Aorta	0	1
Lung	0	1
Skin	0	1